Plant mortality varies with arbuscular mycorrhizal fungal species identities in a self-thinning population

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Because arbuscular mycorrhizal fungal (AMF) species differ in stimulating the growth of particular host plant species, AMF species may vary in their effects on plant intra-specific competition and the self-thinning process. We tested this hypothesis using a microcosm experiment with *Medicago sativa* L. as a model plant population and four AMF species. Our results showed that the AMF species *Glomus diaphanum* stimulated host plant growth more than the other three AMF species did when the plants were grown individually. *Glomus diaphanum* also induced the highest rate of mortality in the self-thinning plant populations. We also found a positive correlation between mortality and growth response to colonization. Our results demonstrate that AMF species can affect plant mortality and the self-thinning process by affecting plant growth differently.

Keywords: arbuscular mycorrhizal fungi; *Medicago sativa* L.; plant mortality; plant self-thinning

1. INTRODUCTION

It has been widely recognized that arbuscular mycorrhizal fungi (AMF) can influence plant interactions and plant population dynamics [1]. Intra-specific competition was higher with AMF than without in non-self-thinning plant populations [1,2]. In self-thinning plant populations, AMF were reported to have little effect on plant competition [3]. Because different AMF species often vary in host plant growth stimulation [4], and because mycorrhizal fungal communities often vary in species composition, the influence of AMF identity on plant–plant interactions warrants elucidation if we are to understand the role of diverse mycorrhizal fungal communities in nature. Although Hoeksema [5] has found that plant intra-specific competition varied with different ectomycorrhizal fungal species, there is no published examination of the effect of different AMF identities on self-thinning.

Self-thinning is a phenomenon whereby as a crowded stand of plants develops, some individuals die, while others grow. In a self-thinning population, mortality originates from intra-specific competition for limiting resources (such as light). In theory, the

larger the plants are, the more intensive the competition would be for limiting resources. Thus, AMF identity could influence mortality through influencing host plant size. Here we used a legume plant *Medicago sativa* L. and four common indigenous AMF species to test this hypothesis.

2. MATERIAL AND METHODS

(a) Experimental design

The soil used here is the same as that in a previous study [6]. The legume plant *M. sativa* L. was used as model plant because it strongly depends on AMF [7]. Four indigenous AMF species (*Glomus versiforme*, *Glomus diaphanum*, *Glomus geosporum* and *Glomus etunicatum*) [8] were used in this experiment.

AMF species was one factor in this experiment, and plant density was another. Microcosms (14 × 13 × 15 cm) were filled with 3 kg of soil. Spores of the four different AMF species were inoculated into each microcosm ($n = 8$ microcosms per AMF species). For the non-mycorrhizal control ($n = 8$ microcosms), washing filtrate and the sterilized leftover of the inoculum were added to correct for possible differences in the microbial community between mycorrhizal and non-mycorrhizal treatments.

We set up two densities in this study. For the low density, one seedling of *M. sativa* was kept in each microcosm (LD). For the high density, 250 seeds of *M. sativa* were sown for each microcosm. Before sowing, a thin layer of soil was sieved over the microcosms to provide a smooth surface to minimize spatial heterogeneity. Seeds were mixed with sand and sown with a sieve to achieve a random spatial pattern, with a density equal to 10 000 plants per square metre (HD). Within each AMF species identity treatment, four replicates were used for the low density and high density, respectively, achieving a total of 40 microcosms. All microcosms were arranged in a completely randomized design under greenhouse conditions from April to September in 2008 (see electronic supplementary material for more detailed information about experimental design).

Plants were harvested 160 days after seeding, when high-density plant populations had experienced a self-thinning process and most of the large individuals had flowered. Mortality was apparently randomly distributed across the area of the high-density populations. Above-ground plant biomass and surviving plant numbers in high density were recorded. Root systems were collected and cleaned for AM colonization detection by the gridline intersection method [9].

(b) Data analysis

Mortality was calculated as: mortality (%) = (initial plant density – final plant density)/initial plant density × 100%.

For the high-density treatment, as one of the four replicates was destroyed during the experiment, we used the data of three replicates for the analysis. For the low-density and high-density datasets separately, one-way ANOVA was performed to test for the effects of AMF species identity on AM colonization rate, shoot biomass, plant number and mortality in DPS software [10]. Least significant difference at the 5 per cent alpha level was used for comparison between treatments. Normality and homogeneity of variances were tested before ANOVA. We performed linear regression modelling to test the relationship between shoot biomass and colonization rate with the data from the four mycorrhizal treatments (non-mycorrhizal control data were not included) in both densities. The relationship between mortality and mean shoot biomass was also tested in the high density with all the five mycorrhizal treatment data included. We also used linear regression to test the relationship between mean shoot biomass for each species treatment in the high density and mean shoot biomass for each species identity in low density.

3. RESULTS

At low density, AM colonization and shoot biomass were significantly different among AMF treatments (table 1), including differences in shoot mass among different AMF species. In the non-mycorrhizal control, no mycorrhizal colonization was detected. At high density, mortality and mean shoot biomass varied with AMF identities (table 1). Plant growth and mortality were highest with *G. diaphanum* (table 1). *Glomus versiforme* had no significant effect on plant growth and mortality when compared with non-mycorrhizal control (table 1).

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Table 1. Parameters of *Medicago sativa* L. in the experiment under two densities. (Data are mean (s.d.).) NM, non-mycorrhizal control; G.d., *Glomus diaphanum*; G.e., *Glomus etunicatum*; G.v., *Glomus versiforme*; G.g., *Glomus geosporum*. None means no AM colonization was detected in non-mycorrhizal control, and data were not included in ANOVA. Treatments indicated with different letters are significantly different at $\alpha = 0.05$. F and p represent F values and p -values of ANOVA tests for differences under different AM treatments.

	LD		HD		
	colonization (%)	shoot biomass (g)	colonization (%)	mean shoot biomass (g)	mortality (%)
NM	none	1.075 (0.119) ^c	none	0.016 (0.001) ^d	32.78 (2.54) ^d
G.d.	50.24 (3.27) ^a	3.100 (0.324) ^a	44.70 (1.10) ^{ab}	0.138 (0.006) ^a	79.63 (4.72) ^a
G.e.	31.34 (2.00) ^b	2.000 (0.129) ^b	30.07 (3.49) ^c	0.050 (0.003) ^b	45.00 (10.60) ^c
G.v.	48.32 (5.18) ^a	1.325 (0.144) ^c	50.02 (3.35) ^a	0.017 (0.001) ^d	25.00 (4.19) ^d
G.g.	25.79 (2.59) ^c	2.138 (0.170) ^b	39.10 (1.61) ^b	0.036 (0.005) ^c	62.96 (3.70) ^b
F	67.71	49.36	10.64	190.32	43.24
p	0.0001	0.0001	0.0036	0.0001	0.0001

Figure 1. The relationship between mortality and mean shoot biomass in self-thinning populations of *Medicago sativa* L. Values of r^2 ($=0.6900$) and p (<0.0001) were estimated from a linear regression model. Right-faced triangle, NM; filled triangle, *G. versiforme*; filled circles, *G. etunicatum*; open circles, *G. geosporum*; filled squares, *G. diaphanum*.

In the high-density population, a significantly positive relationship was found between mortality and mean shoot biomass (figure 1). The fungus *G. diaphanum* promoted plant growth more than the other fungi whenever the host plant was grown individually (table 1 and figure 2) or in self-thinning populations (table 1), and plant mortality was the highest when inoculated with this fungus (table 1 and figure 1). A significantly positive relationship was also found between mean shoot biomass in high densities and mean shoot biomass when grown individually (figure 2). The relationship between shoot biomass and colonization rate was not significant under both densities (for LD: $r^2 = 0.031$, $p = 0.511$; for HD: $r^2 = 0.0016$, $p = 0.901$).

4. DISCUSSION

The effects of host–fungi partnerships vary with AMF species. For example, AMF species vary in their ability to acquire resources below ground [11] and to stimulate host plant growth [4]. These differential growth responses of plant species to AMF species

Figure 2. The relationship between the mean shoot biomass in self-thinning populations of *Medicago sativa* L. and the shoot biomass when *M. sativa* were grown individually. Data shown were the average of the replicates. Values of r^2 ($=0.8690$) and p ($=0.0209$) were estimated from a linear regression model. LD means low density (plants were grown individually), and HD means high density.

may shift the balance of interspecific plant interactions and influence plant community structure [4,12,13]. In our experiment, we found that AMF identities also affected plant mortality in a self-thinning experimental population of *M. sativa*. Plant mortality during the self-thinning process is often explained by intra-specific competition for limiting resources, usually light or space. AMF have been shown to increase mortality by increasing host plant growth and branching, and have no effect on mortality when plant growth was not affected [3]. Hoeksema [5] also reported that plant intra-specific competition was stronger when colonized with the ectomycorrhizal fungus that caused a higher plant growth rate. Here we found that AMF identities affected plant mortality differently, by affecting plant growth differently. For example, *G. diaphanum* promoted plant growth more than the other mycorrhizal fungi did when plants were grown individually (table 1), and this growth stimulation generated a higher mortality rate (table 1) during self-thinning, probably owing to higher shoot competition. In contrast, *G. versiforme*

did not influence mortality when compared with non-mycorrhizal controls, as it had no effect on plant growth.

As smaller individuals usually die first during self-thinning, variable AMF species effects on mortality could have long-term consequences for plant population dynamics. When mycorrhizal fungi promote self-thinning, they are selecting for plants that are particularly responsive to mycorrhizal colonization. Therefore, it seems that the mycorrhizal fungi that promote growth the most are imposing more selection than fungi that do not promote growth as much. When a host plant population encounters an AMF community with a preferred dominant species, plant individuals will grow faster than with an AMF community dominated by species that are not preferred. In the former population, smaller individuals would tend to suffer more seriously from competition pressure, and the onset of self-thinning would appear earlier. It is also clear that these plant populations would suffer the most substantial mortality even with growing of larger individuals. This process would produce fewer, larger individuals within these plant populations. Sometimes small plants are small owing to random events, but sometimes small plants are small owing to genetic variation [14]. This suggests that, in a community where *G. diaphanum* was dominant, natural selection might occur over time for the genes that confer on the plants greater responsiveness to colonization. However, in a community where *G. versiforme* was dominant, there would be no selection for the genes that confer greater responsiveness to colonization.

Distribution patterns (random or uniform) can strongly impact plant interactions [15]. In our study, we examined these effects of AMF identities in a random distribution pattern. It is worth elucidating the role of AMF identities on plant interactions under a uniform pattern of plant distribution, as this may help explain the role of AMF in agricultural systems [16], where plant distribution is usually in a uniform pattern or in a regular row [17].

As only four AM fungi and one self-thinning density were used in this experiment, these results need to be corroborated by further studies with more AMF species and a wider range of plant densities. Overall, our study showed that different AMF identities induced different self-thinning rates through influencing plant growth, and that AMF species that promoted plant growth most are also those that increased mortality the most. These results provided direct evidence that variation in AMF species effects on plant growth can induce variation in plant population dynamics, advancing our knowledge of how AMF community composition may affect plant populations.

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- 1 Koide, R. T. & Dickiel, I. A. 2002 Effects of mycorrhizal fungi on plant populations. *Plant Soil* **244**, 307–317. (doi:10.1023/A:1020204004844)

- 2 Facelli, E. & Facelli, J. M. 2002 Soil phosphorus heterogeneity and mycorrhizal symbiosis regulate plant intra-specific competition and size distribution. *Oecologia* **133**, 54–61. (doi:10.1007/s00442-002-1022-5)
- 3 Shumway, D. L. & Koide, R. T. 1995 Size and reproductive inequality in mycorrhizal and nonmycorrhizal populations of *Abutilon theophrasti*. *J. Ecol.* **83**, 613–620.
- 4 van der Heijden, M. G. A., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. & Sanders, I. R. 1998 Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**, 69–72. (doi:10.1038/23932)
- 5 Hoeksema, J. D. 2005 Plant–plant interactions vary with different mycorrhizal fungus species. *Biol. Lett.* **1**, 439–442. (doi:10.1098/rsbl.2005.0381)
- 6 Zhang, Q., Yang, R. Y., Tang, J. J. & Chen, X. 2008 Competitive interaction between the invasive *Solidago canadensis* and native *Kummerowia striata* in lead contaminated soil. *Bot. Stud.* **49**, 385–391.
- 7 Zhang, Q., Xu, L. M., Tang, J. J., Bai, M. E. & Chen, X. In press. Arbuscular mycorrhizal mediation of biomass–density relationship of *Medicago sativa* L. under two water conditions in a field experiment. *Mycorrhiza*. (doi:10.1007/s00572-010-0331-5)
- 8 Zhang, Q., Yang, R. Y., Tang, J. J., Yang, H. S., Hu, S. J. & Chen, X. 2010 Positive feedback between mycorrhizal fungi and plants influences plant invasion success and resistance to invasion. *PLoS ONE* **5**, e12380. (doi:10.1371/journal.pone.0012380)
- 9 Giovannetti, M. & Mosse, B. 1980 An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots. *New Phytol.* **84**, 489–500. (doi:10.1111/j.1469-8137.1980.tb04556.x)
- 10 Tang, Q. Y. & Feng, M. G. 2002 *DPS data processing system for practical statistics*. Beijing, China: Science Press.
- 11 Jakobsen, I., Abbott, L. K. & Robson, A. D. 1992 External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. I. Spread of hyphae and phosphorus inflow into roots. *New Phytol.* **120**, 371–380. (doi:10.1111/j.1469-8137.1992.tb01077.x)
- 12 Scheublin, T. R., van Logtestijn, R. S. P. & van der Heijden, M. G. A. 2007 Presence and identity of arbuscular mycorrhizal fungi influence competitive interactions between plant species. *J. Ecol.* **95**, 631–638. (doi:10.1111/j.1365-2745.2007.01244.x)
- 13 Vogelsang, K. M., Reynolds, H. L. & Bever, J. D. 2006 Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytol.* **172**, 554–562. (doi:10.1111/j.1469-8137.2006.01854.x)
- 14 Weiner, J. 1990 Asymmetric competition in plant populations. *Trends Ecol. Evol.* **5**, 360–364. (doi:10.1016/0169-5347(90)90095-U)
- 15 Weiner, J., Stoll, P., Muller-Landau, H. & Jasentuliyana, A. 2001 The effect of density, spatial pattern, and competitive symmetry on size variation in simulated plant populations. *Am. Nat.* **158**, 438–450. (doi:10.1086/321988)
- 16 Rinaudo, V., Bàrberi, B., Giovannetti, M. & van der Heijden, M. G. A. 2010 Mycorrhizal fungi suppress aggressive agricultural weeds. *Plant Soil* **333**, 7–20. (doi:10.1007/s11104-009-0202-z)
- 17 Weiner, J., Andersen, S. B., Wille, W. K.-M., Griepentrog, H.-W. & Olsen, J. M. 2010 Evolutionary agroecology: the potential for cooperative, high density, weed suppressing cereals. *Evol. Appl.* **3**, 473–479. (doi:10.1111/j.1752-4571.2010.00144.x)